Association between Serum Amyloid A Level and White Matter Hyperintensity Burden: a Cross-Sectional Analysis in Patients with Acute Ischemic Stroke

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ABSTRACT

Introduction: This work aimed to determine the potential link between white matter hyperintensity (WMH) burden and serum amyloid A (SAA) level in patients with acute ischemic stroke.

Methods: Consecutive patients with acute large artery atherosclerosis (LAA) stroke between April 2021 and May 2022 were included. WMH volumes (periventricular, deep, and total) were measured using the Fazekas score and a semi-automated volumetric analysis on fluid-attenuated inversion recovery–magnetic resonance imaging. The burdens of WMH were scored to assess the dose-dependent association between SAA and WMH volume. Multivariate regression and a two-piecewise linear regression model were used to evaluate whether SAA levels are an independent predictor of WMH, and to discover the threshold effect or saturation effect of SAA levels with respect to WMH volume.

Results: The mean age of patients was 63.2 ± 11.5 years, with 65.9% men. The median SAA level was 3.93 mg/L and the total WMH volume of 6.86 cm^3. In the multivariable analysis, SAA remained an independent predictor of total WMH volume [$\beta = 0.82$, 95% confidence interval (CI) = 0.49–1.07, $p < 0.001$], periventricular WMH volume (adjusted $\beta = 0.76$, 95% CI = 0.46–1.07, $p < 0.001$), and deep WMH volume (adjusted $\beta = 0.26$, 95% CI = 0.06–0.45, $p = 0.011$) after controlling for confounders. Furthermore, SAA levels were associated with periventricular Fazekas score, deep Fazekas score, and Fazekas grades. Threshold effect and saturation effect analyses demonstrated a nonlinear relationship between SAA levels and periventricular white matter hyperintensity (PVWMH) volumes, with SAA levels (2.12–19.89 mg/L) having significant dose-dependent relationships with periventricular WMH volumes (adjusted $\beta = 1.98$, 95% CI = 1.12–2.84, $p < 0.001$).

Peng Zhang and Rongrong Han have contributed equally to this work.

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Conclusion: SAA level ranging from 2.12 to 19.89 mg/L is dose-dependently associated with periventricular WMH development. These findings point the way forward for future research into the pathophysiology of WMH.

Keywords: White matter hyperintensity; Serum amyloid A; Acute ischemic stroke

Key Summary Points
Traditional vascular risk factors explain only up to 2% of the white matter hyperintensity (WMH) variance; therefore, elucidating the associated risk factors is important.
We hypothesized that serum amyloid A (SAA) with pro-inflammatory and pro-atherosclerosis activity is associated with the formation and development of WMH.
SAA is a predictor of WMH, and the level ranging from 2.12 to 19.89 mg/L is dose-dependently associated with periventricular WMH development.
These findings point the way forward for future research into the pathophysiology of WMH.

INTRODUCTION

White matter hyperintensity (WMH)—areas of high intensity in the periventricular and deep cerebral white matter on T2-weighted (T2W) or T2 fluid-attenuated inversion recovery images is a common feature of brain magnetic resonance imaging (MRI), particularly in the elderly with vascular risk factors and cerebrovascular disease [1]. Although the pathophysiological mechanism of WMH is not fully understood and may be complex, several researchers have attempted to explain the above phenomenon using data such as atherosclerosis, widespread hypoperfusion, and endothelial dysfunction [1–4]. Nevertheless, the mechanism underlying the above finding is unknown. Evidence indicates that hypertension, diabetes, smoking, and homocysteine levels are major risk factors for WMH [5]. However, WMH has a significant "non-vascular" component, and standard vascular risk factors explain only up to 2% of the WMH variance [6]. Moreover, restricted randomized control trial data on the influence of traditional risk factors reduction on WMH progression have yielded inconsistent results [7–9]. As a result, there is an urgent need to identify additional variables that will add to our understanding of WMH etiology.

Serum amyloid A (SAA) is a small protein family (103–104 amino acids) with significant sequence homology that is increased in acute-phase response and persistently elevated in chronic inflammatory situations [10, 11]. In a recently published study, SAA was revealed as a novel independent predictor of infection after stroke [12]. Epidemiologic studies have demonstrated robust associations of elevated SAA with increased risk of atherosclerosis, rheumatoid arthritis, chronic obstructive pulmonary disease, and tumor [13–16]. Studies have further linked higher SAA levels to Alzheimer's dementia and cardiovascular disease, implying that endothelial dysfunction is a primary cause of disease [17–20]. Endothelial dysfunction has been investigated as a potential cause of WMH [1, 3]. Thus, increased SAA levels may be linked to the formation and development of WMH. However, most previous studies only looked at the relationships between SAA levels and cardiovascular disease in individuals with varying characteristics [17–19], and epidemiological data showed that higher SAA is associated with cerebrovascular disease mortality [21]. Few studies have investigated the possible link between SAA levels and cerebrovascular diseases, particularly in WMH of suspected vascular origin.

The purpose of this study was to determine the potential link between the SAA level and WMH in patients with acute ischemic stroke. The SAA levels among individuals with WMH were also analyzed based on their burden to find out more about the underlying pathomechanisms of WMH.

METHODS

Patients and Population

SAA levels may vary in participants with different types of ischemic stroke. To eliminate the impact of this confounding factor, we took it into account and limited the study population to patients with large artery atherosclerosis (LAA) stroke. Consecutive patients with LAA stroke within 72 h after symptom onset between April 2021 and May 2022 were recorded from the electronic medical record in our center ($n = 544$). Registry discharge diagnoses of acute LAA stroke are > 99% correct, while diagnoses of acute LAA stroke were individually validated by two independent neurologists reviewing medical records. Inclusion criteria were as follows: (1) clinical symptoms, signs, and cranial MRI + brain magnetic resonance angiography (MRA) examination confirmed acute LAA stroke; (2) without intravenous thrombolysis and endovascular treatment due to exceeding the therapeutic time window; and (3) age of the patients ranged from 30 to 80 years. Exclusion criteria were as follows: (1) those with a previous history of Alzheimer's disease, Parkinson's disease, psychiatric illness, cerebral amyloid angiopathy, chronic obstructive pulmonary disease, or head injury; (2) complicated with functional failure of heart, kidney, lung, liver, or other important organs; (3) complicated with neoplastic, inflammatory, or autoimmune diseases; (4) complicated with stroke-associated infection; and (5) clinical data collection was incomplete. Finally, participants with stroke-associated infection ($n = 53$), tumor or autoimmune disease ($n = 4$), and insufficient MRI image ($n = 21$) or SAA missing ($n = 287$) were excluded from the trial. The final analysis included 179 acute LAA stroke subjects having data on brain MRI and SAA inspection findings (Fig. 1). The admission screenings included broad evaluations, including SSA level, brain MRI, brain magnetic resonance angiography (MRA), and laboratory examinations.
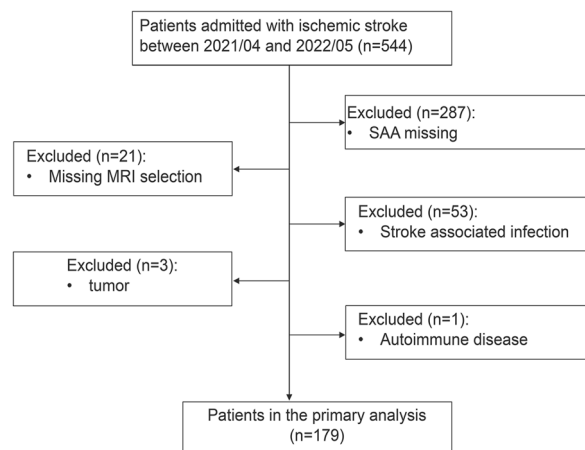

Fig. 1 Study flowchart. *MRI* magnetic resonance imaging; *SAA* Serum amyloid A

Standard Protocol Approvals, Registrations, and Patient Consent

Ethics approval was obtained from the Scientific Research Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China) (approval no. 2022C075). All participants provided written informed consent regarding the use of their clinical data and biological samples for scientific research. This study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments.

Clinical Assessment

Patient demographics and clinical variables, major vascular risk factors, and laboratory factors were all obtained retrospectively. Laboratory examinations including fasting plasma glucose level, lipid profile, creatinine, homocysteine, and SAA level were also evaluated after 12 h of fasting. To detect SAA levels, venous samples were taken from patients within 24–72 h of onset and were immediately separated by centrifugation ($1920g$ for 15 min). The levels of SAA in serum were then determined using an immunofluorescent antibody method within 2 h of collection. The minimal detection concentration of SAA is 2.0 mg/L, and concentrations less than 2.0 mg/L were statistically

analyzed using half of the lower limit values. All clinical and laboratory factors of all participants were comprehensively assessed.

Imaging Assessment

All image acquisitions were performed on 3.0-T magnetic resonance scanners (Philips Ingenia CX, Best, the Netherlands). The following broad MRI acquisitions were acquired: T1-weighted images [repetition time (TR)/echo time (TE) = 250/2.3 ms (ms)], T2-weighted images (TR/TE = 4000/102 ms), T2 fluid-attenuated inversion recovery images (TR/TE = 4800/310 ms), and three-dimensional time-of-flight MRA images (TR/TE = 20/3.45 ms, slice thickness = 1.0 mm). With the exception of the time-of-flight MRA images, the basic slice thickness was 5 mm in the axial plane.

WMHs were identified as variable-sized signal abnormalities in the white matter of the brain that produce a hyperintense signal on FLAIR images [1]. A MIPAV software package (NIH, Bethesda, MD, USA) was used to quantitatively measure the total white matter hyperintensity (TWMH), deep white matter hyperintensity (DWMH), and periventricular white matter hyperintensity (PVWMH) volumes using a previously described semiautomated volumetric analysis method [22]. The deep and periventricular sub-segmentation of WMH was based on the discontinuity of the DWMH, which is not touching the PVWMH, as opposed to the PVWMH, which is in direct contact with the ventricles. PVWMH was defined as WMH in contact with the ventricles that could be followed on two slices above the ventricles. Above this level, WMH was confirmed as DWMH [23]. To avoid the confounding effect caused by the presence of focal white matter damage owing to ischemic stroke, we measured only WMH from the unaffected hemisphere and doubled it to determine the final WMH volume in all patients, which was a validated procedure with strong interhemispheric correlation in WMH severity [24]. We also used a manual rating method based on the Fazekas scale to rate WMH volume [25]. All clinical data, as well as other metrics, were kept hidden from the image analysts. All imaging assessments were reviewed by two well-trained neurologists, and disagreements were settled through consensus.

Statistical Analysis

Continuous variables with normal distributions were expressed as mean $\pm$ SD; those with abnormal distributions were presented as median [interquartile range (IQR)]. Continuous variables with skewed distributions were transformed into a log scale or squared root scale. In univariable analyses, we used simple regression analyses for TWMH, DWMH, and PVWMH volume to assess whether SAA is a potential predictor of WMH. In the univariable analyses, variables with p-values < 0.10, as well as for creatinine and homocysteine, were included in the multivariable regression analyses. Next, relationships between TWMH, PVWMH, and DWMH volumes and SAA levels were investigated using multivariable regression models with crude and adjusted β-values and 95% CI in multivariable regression models.

To assess the dose-dependence of the relationships between SAA and WMH volumes, we compared mean SAA levels among individuals with varying loads of TWMH, PVWMH, and DWMH volumes assessed using both the Fazekas score and a semiautomated volumetric analysis. Next, we used a smoothing function and a two-piecewise linear regression model to examine the threshold effect or saturation effect of the log SAA on TWMH, PVWMH, and DWMH volumes. The threshold level or saturation level (e.g., turning point) was confirmed using trial and error, which included selecting turning points along a predefined interval and then selecting turning points that produced the highest model likelihood. This step is relevant to R programming, an additional file shows this in more detail (see the appendix in the electronic supplementary material).

To examine the characteristics of groups with high and low SAA levels, we used the median value to divide the whole cohort into a higher SAA group and a lower SAA group. Next, the Student's t-test or the Mann–Whitney U test was used to evaluate continuous variables, and

Table 1 Baseline characteristics of the cohort ($n = 179$)

	Total
Age, years	63.2 ± 11.5
Sex, male	118 (65.9)
Hypertension	105 (58.7)
Diabetes	44 (24.6)
Hyperlipidemia	35 (19.6)
Ischemic heart disease	37 (20.7)
Stroke	64 (35.8)
Current smoking	72 (40.2)
Current alcohol	80 (44.7)
Antiplatelet medication	46 (25.7)
Statins	37 (20.7)
Systolic blood pressure, mmHg	145 (134–158)
Diastolic blood pressure, mmHg	85 (75–95)
Glucose, mmol/L	5 (4.6–5.9)
Total cholesterol, mmol/L	4.16 ± 1.11
Triglyceride, mmol/L	1.17 (0.89–1.60)
Homocysteine, µmol/L	11.7 (9.7–14.2)
SAA, mg/L	3.93 (2.63–7.29)
Infarction volume, cm^3	0.90 (0.45–2.67)
Total WMH volume, cm^3	6.86 (0.73–16.98)
DWMH volume, cm^3	0.80 (0–4.02)
PVWMH volume, cm^3	6.01 (0.60–12.72)
Fazekas score	
DWMH	
0	91 (50.9)
1	38 (21.2)
2	26 (14.5)
3	24 (13.4)
PVWMH	
0	41 (22.9)
1	64 (35.8)
2	43 (24.0)

Table 1 continued

	Total
3	31 (17.3)

Data represent mean $\pm$ SD, median (interquartile range) or n (%)

SAA serum amyloid A protein, *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity

the chi-square test or Fisher exact test were used to analyze categorical variables.

SAA level is likely to be affected by the size of infarction volume and the severity of ischemic stroke. To avoid bias caused by differences in the size of infarction volume and the severity of ischemic stroke, we also conducted the following two sensitivity analyses of the study population. Given that all of the participants suffered mild-to-moderate ischemic stroke, patients with a National Institutes of Health Stroke Scale (NIHSS) score > 3 were selected as the study population for sensitivity analysis. Similarly, patients with cerebral infarct volume > 0.9 cm^3 were also included in another sensitivity analysis using a median infarct volume of 0.9 cm^3 as the cut-off point.

We used multiple imputation, based on five replications and a chained equation approach method in the R MI procedure, to account for missing data. The results of data interpolation were consistent with that of the original data.

All statistical analyses in the study were performed using EmpowerStats version 4.0 (X&Y Solutions, inc. Boston, MA, USA) and R software. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The final study population included 179 participants (mean age, 63.2 ± 11.5 years; male sex, 65.9%). The median SAA level was 3.93 (2.63–7.29) mg/L. The median volume of TWMH, PVWMH, and DWMH lesions were 6.86 (0.73–16.98) cm^3, 6.01 (0.60–12.72) cm^3, and 0.80 (0–4.02) cm^3, respectively. The baseline characteristics of the cohort are displayed in

Table 2 Baseline characteristics of patients with low and high SAA levels

	Lower SAA group, ≤ 3.93 mg/L	Higher SAA group, > 3.93 mg/L	*p*-Value
No	91	88	
Age, years	62.09 ± 10.68	64.44 ± 12.23	0.171
Sex, male	63 (69.23)	55 (62.50)	0.342
Body mass index	24.35 ± 3.46	25.15 ± 3.58	0.130
Hypertension	50 (54.95)	55 (62.50)	0.305
Diabetes	23 (25.27)	21 (23.86)	0.826
Hyperlipidemia	20 (21.98)	15 (17.05)	0.405
Ischemic heart disease	18 (19.78)	19 (21.59)	0.765
Stroke	34 (37.36)	30 (34.09)	0.648
Current smoking	40 (43.96)	32 (36.36)	0.300
Current alcohol	43 (47.25)	37 (42.05)	0.484
Antiplatelet medication	20 (21.98)	26 (29.55)	0.247
Statins	19 (20.88)	18 (20.45)	0.944
Systolic blood pressure, mmHg	144 (130–156)	146 (135–159)	0.225
Diastolic blood pressure, mmHg	83 (75–96)	86 (76–95)	0.288
Glucose, mmol/L[a]	1.59 (1.50–1.70)	1.65 (1.55–1.84)	0.244
Total cholesterol, mmol/L	4.20 ± 1.00	4.12 ± 1.22	0.633
Triglyceride, mmol/L[a]	0.15 (−0.20 to 0.47)	0.17 (−0.07 to 0.47)	0.350
Homocysteine, μmol/L[a]	2.51 (2.28–2.71)	2.41 (2.20–2.54)	0.008
Infarction volume, cm^3[a]	−0.21 (−0.81 to 1.00)	−0.00 (−0.77 to 0.81)	0.372
TWMH volume, cm^3[b]	1.64 (0.00–3.39)	3.43 (1.98–5.04)	< 0.001
DWMH volume, cm^3[b]	0.00 (0.00–1.76)	1.59 (0.00–2.31)	< 0.001
PVWMH volume, cm^3[b]	1.38 (0.00–2.86)	2.96 (1.94–4.38)	< 0.001
Fazekas score			
DWMH			0.010
0	56 (61.54)	35 (39.77)	
1	19 (20.88)	19 (21.59)	
2	8 (8.79)	18 (20.45)	
3	8 (8.79)	16 (18.18)	
PVWMH			0.002
0	30 (32.97)	11 (12.50)	
1	33 (36.26)	31 (35.23)	
2	19 (20.88)	24 (27.27)	

Table 2 continued

	Lower SAA group, ≤ 3.93 mg/L	Higher SAA group, > 3.93 mg/L	*p*-Value
3	9 (9.89)	22 (25.00)	

SAA serum amyloid A, *TWMH* total white matter hyperintensity, *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity

Data represent mean ± SD, median (interquartile range) or *n* (%)

[a]These variables were transformed to log scale

[b]These variables were transformed to a square root scale

Table 1. The higher SAA group did not vary from the lower SAA group base on male sex, hypertension, diabetes, current smoking, or a history of a previous stroke, but had higher levels of TWMH, PVWMH, and DWMH volume (Table 2).

After controlling for relevant confounders (age, sex, hypertension, diabetes, hyperlipidemia, history of previous stroke, current smoking, antiplatelet medication, statins, total cholesterol, creatinine, and homocysteine), SAA remained an independent predictor of total WMH volume [β = 0.82; 95% confidence interval (CI) = 0.49–1.16, $p < 0.001$], periventricular WMH volume (adjusted β = 0.76; 95% CI = 0.46–1.07, $p < 0.001$), and deep WMH volume (adjusted β = 0.26; 95% CI = 0.06–0.45, $p = 0.011$; Tables 3 and 4).

The link between SAA levels and WMH loads was also examined, and correlations were found between SAA levels and the modified Fazekas score in both periventricular ($p = 0.004$, p for trend < 0.023) and deep ($p = 0.001$, p for trend $= 0.008$) areas. A similar trend was demonstrated between SAA levels and the Fazekas grade in total WMH burden ($p = 0.001$, p for trend < 0.008; Fig. 2).

Adjusted smoothed plots suggested nonlinear relationships between SAA and TWMH, PVWMH, and DWMH volumes. There were two inflection points between SAA levels and PVWMH volume. With a log SAA < 0.75 (SAA < 2.12 mg/L) and a log SAA ≥ 1.99 (SAA ≥ 19.89 mg/L), the predicted dose-response curve was consistent with a horizontal line. In other words, the relationships between the SAA levels and PVWMH volumes were not statistically significant ($p = 0.074$ and 0.612,

respectively). However, SAA levels ranging from 2.12 to 19.89 mg/L demonstrated a significant correlation between SAA and the PVWMH volume in a dose-dependent manner (adjusted β = 1.98; 95% CI = 1.12–2.84, $p < 0.001$). Additionally, the TWMH volume increased with the SAA level up to the turning point log SAA < 2.25 (SAA < 9.49 mg/L) (adjusted β = 1.14; 95% CI = 0.70–1.58, $p < 0.001$). When the log SAA level was ≥ 2.25 (SAA ≥ 9.49) mg/L, the level of SAA was not associated with the TWMH volume (adjusted β = −0.10; 95% CI = −0.98 to 0.78, $p = 0.825$). Our two-piecewise linear regression model also revealed a significant correlation between SAA and the DWMH volume (adjusted β = 0.46; 95% CI = 0.19–0.74, $p = 0.001$) at log SAA level < 2.00 (SAA < 7.39 mg/L) (Fig. 3, Table 5).

In two sensitivity analyses, SAA remained an independent predictor of TWMH volume and PWMH volume in patients with NIHSS scores > 3 or ≤ 3. Similarly, SAA level was still associated with TWMH volume in both the study population with infarct volume > 0.9 cm³ or ≤ 0.9 cm³. In general, SAA level was hardly affected by the severity of ischemic stroke or the size of infarct volume (see Table S1 in the electronic supplementary material for details).

DISCUSSION

In this population-based study, we evaluated the potential association of SAA levels with WMH in an acute ischemic stroke group. These relationships were consistent in dose-dependent studies of WMH volumes within a particular range. As a result, these findings may

Table 3 Simple regression analyses between possible predictors and the squared root white matter hyperintensity volume[b]

	TWMH volume[b]		PVWMH volume[b]		DVWMH volume[b]	
	β (95% CI)	p-Value	β (95% CI)	p-Value	β (95% CI)	p-Value
Age	0.04 (0.01, 0.07)	0.003	0.04 (0.01, 0.06)	0.003	0.02 (0.00, 0.03)	0.011
Sex, male	0.23 (−0.44, 0.90)	0.498	0.11 (−0.49, 0.72)	0.711	0.18 (−0.18, 0.54)	0.336
BMI	−0.03 (−0.12, 0.06)	0.474	−0.04 (−0.12, 0.04)	0.359	−0.01 (−0.06, 0.04)	0.640
Hypertension	0.68 (0.05, 1.32)	0.037	0.61 (0.04, 1.18)	0.038	0.26 (−0.09, 0.60)	0.147
Diabetes	−0.65 (−1.38, 0.08)	0.084	−0.57 (−1.23, 0.09)	0.091	−0.39 (−0.78, 0.01)	0.055
Hyperlipidemia	1.08 (0.29, 1.86)	0.008	0.98 (0.28, 1.69)	0.007	0.51 (0.09, 0.94)	0.020
IHD	0.65 (−0.12, 1.43)	0.101	0.51 (−0.19, 1.21)	0.152	0.35 (−0.07, 0.78)	0.101
Stroke	1.36 (0.73, 1.99)	< 0.001	1.19 (0.62, 1.76)	< 0.001	0.73 (0.38, 1.07)	< 0.001
Current smoking	1.36 (0.73, 1.99)	0.011	−0.71 (−1.28, −0.14)	0.016	−0.39 (−0.74, −0.05)	0.027
Current alcohol	−0.61 (−1.24, 0.02)	0.059	−0.57 (−1.14, −0.00)	0.050	−0.23 (−0.58, 0.11)	0.189
Antiplatelet medication	1.14 (0.43, 1.84)	0.002	1.04 (0.40, 1.67)	0.002	0.55 (0.16, 0.93)	0.006
Statins	1.34 (0.59, 2.10)	< 0.001	1.19 (0.50, 1.87)	< 0.001	0.72 (0.31, 1.13)	< 0.001
Systolic BP	0.01 (−0.01, 0.03)	0.308	0.01 (−0.01, 0.02)	0.249	0.00 (−0.01, 0.01)	0.575
Diastolic BP	0.00 (−0.02, 0.02)	0.793	0.00 (−0.02, 0.02)	0.887	0.00 (−0.01, 0.01)	0.640
Infarction volume[a]	0.06 (−0.17, 0.30)	0.585	0.07 (−0.13, 0.28)	0.483	−0.01 (−0.14, 0.11)	0.852
Glucose[a]	−0.74 (−1.71, 0.24)	0.140	−0.61 (−1.49, 0.26)	0.171	−0.55 (−1.08, −0.02)	0.043
Total cholesterol	−0.36 (−0.64, −0.08)	0.013	−0.33 (−0.58, −0.07)	0.012	−0.15 (−0.31, −0.00)	0.051
Triglyceride[a]	−0.56 (−1.18, 0.06)	0.080	−0.40 (−0.96, 0.16)	0.165	−0.47 (−0.80, −0.14)	0.006
Creatinine[a]	0.60 (−0.55, 1.75)	0.306	0.52 (−0.51, 1.55)	0.326	0.27 (−0.36, 0.89)	0.402
Homocysteine[a]	0.04 (−0.68, 0.76)	0.910	0.07 (−0.57, 0.72)	0.826	−0.02 (−0.41, 0.38)	0.939
SAA[a]	0.90 (0.58, 1.22)	< 0.001	0.80 (0.51, 1.09)	< 0.001	0.38 (0.20, 0.56)	< 0.001

BMI body mass index, BP blood pressure, CI confidence interval, IHD ischemic heart disease, SAA serum amyloid A protein

[a]These variables were transformed to log scale

[b]These variables were transformed to a square root scale

Table 4 Adjusted β (95% CI) for associations between WMH volume and SAA levels

	SAA[a] levels			
	Model I		Model II	
	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value
PWMH[b] volume	0.76 (0.47, 1.06)	< 0.001	0.76 (0.46, 1.07)	< 0.001
DWMH[b] volume	0.35 (0.16, 0.53)	< 0.001	0.26 (0.06, 0.45)	0.011
TWMH[b] volume	0.85 (0.53, 1.18)	< 0.001	0.82 (0.49, 1.16)	< 0.001

TWMH total white matter hyperintensity, *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity, *SAA* serum amyloid A protein, *CI* confidence interval

[a]SAA levels were transformed to a log scale

[b]These variables were transformed to square root scale. Model I adjusted for sex and age. Model II adjusted for sex, age, hypertension, diabetes, hyperlipidemia, stroke, current smoking;, antiplatelet medication, statins, total cholesterol, creatinine, homocysteine

provide useful information for future research into the pathophysiology of WMH.

The current investigation found that higher SAA levels were independently associated with total WMH, implying that dysregulated SAA may play a role in the etiology of overall WMH. However, the bulk of prior investigations has focused on the association of other plasma inflammatory markers [including, total homocysteine (tHcy), hypersensitive-C reactive protein (hs-CRP), vascular cell adhesion molecule-1 (VCAM-1), E-selection, and P-selection] with total WMH burden [26–28]. Few researchers have focused on the impact of different variables on PVWHM and DWMH. Also, the possible mechanisms by which this occurs are unclear. In this view, we are unsure whether SAA levels differentiate between PVWMH and DWMH. Moreover, academic circles have not

agreed on a unified standard of the definition of PVWMH and DWMH lesions, or on the WMH rating scaling procedure, which may explain the past inconsistency. One widely accepted theory is that PVWMH and DWMH have unique histopathological and etiological features [1, 29, 30]. PVWMH is related to changes in the short penetrating microvessels that end close to larger arterial blood vessels [1, 31–33]. They are thought to be more directly influenced by hypertension and traditional risk factors for stroke [33–35]. DWMH lesion occurs in the subcortical areas, which are primarily supplied by long microvessels, and may be damaged as a result of hypertension and the possibility of subsequent hypoperfusion, which is ischemic in nature [33–36]. In our study, we found that higher SAA levels were associated with higher PVWMH and DWMH volumes. The relationships between SAA levels and DWMH volumes were slightly weakened after controlling for covariates but remained statistically significant. Unlike previous genetic studies that found PVWMH and DWMH to have distinct genetic foundations, implying separate etiologies [37], our findings show that there is no difference in the location of the effect of SAA levels on WMH development. An increasing body of evidence that SAA has many pro-inflammatory and proatherogenic activities may explain this phenomenon [13].

Postmortem testing indicates that PVWMH has a nonischemic discontinuous ependymal loss, loosening of the white matter fibers, and loss of myelin [4, 29, 31], implying that microvascular dysfunction is important in the formation and development of PVWMH. A growing body of research suggests that high SAA levels are both directly and indirectly linked to endothelial dysfunction by stimulating endothelial cell migration and proliferation [38]. To understand the strong association between the SAA level and PVWMH, we proposed different endothelial dysfunction-related theories. One widely accepted cause is the loss of blood–brain barrier (BBB) function. It was shown that increased SAA levels induced the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM1), VCAM1, and E-selectin in human endothelial
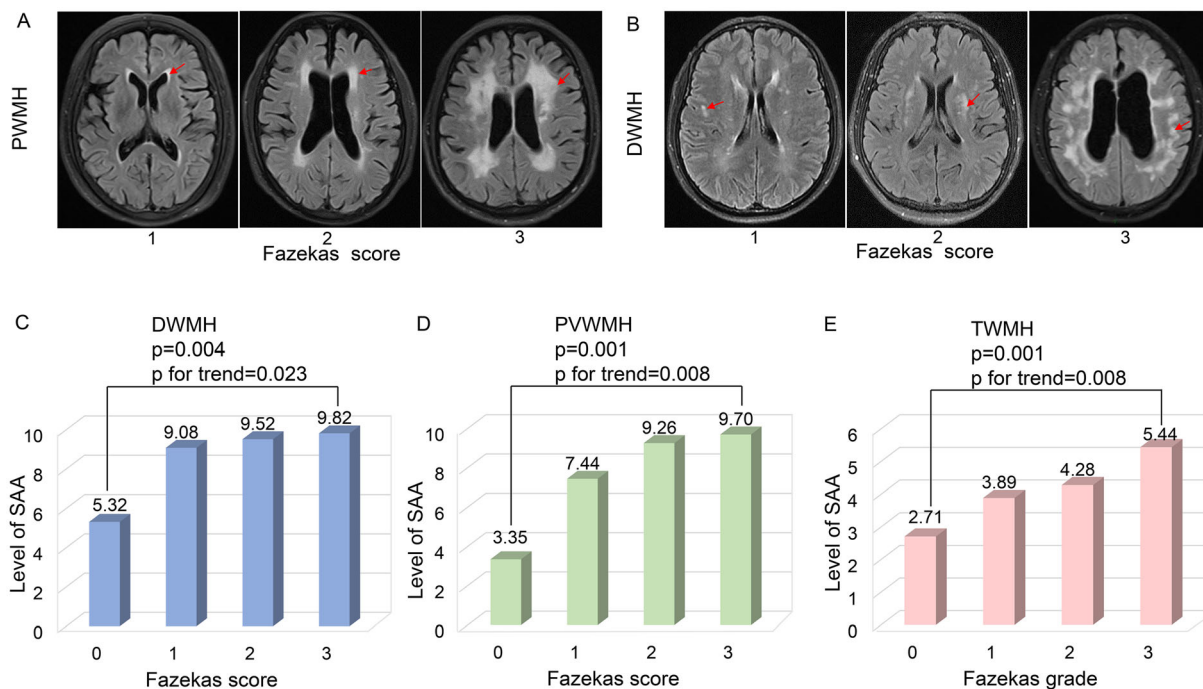
Fig. 2 Distribution of mean values according to the burdens of white matter hyperintensity volume. SAA had positive dose-dependent associations with periventricular Fazekas score ($p = 0.001$, p for trend < 0.008), deep Fazekas score ($p = 0.004$, p for trend $= 0.023$), and Fazekas grade ($p = 0.001$, p for trend $= 0.008$). *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity, *TWMH* total white matter hyperintensity
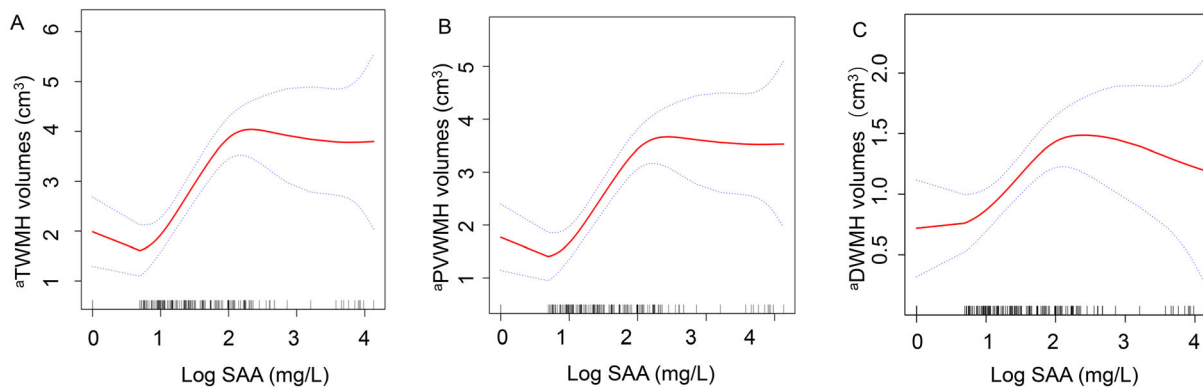

Fig. 3 SAA levels and WMH volumes dose-response relationship. The area between two dotted lines is expressed as a 95% CI. Each point represents the magnitude of the SAA level and is connected to form a continuous line. **A** SAA and TWMH volumes; **B** SAA and PWMH volumes; **C** SAA and DWMH volumes. a: These variables were transformed to square root scale. b: Adjusted for sex, age, hypertension, diabetes, hyperlipidemia, stroke, current smoking, antiplatelet medication, statins, total cholesterol, creatinine, homocysteine. *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity, *TWMH* total white matter hyperintensity

Table 5 Threshold effect or saturation effect analysis of SAA levels on WMH burden using piece-wise linear regression

	Crude β (95% CI)	*p*-Value	Adjusted[b] β (95% CI)	*p*-Value
TWMH[a]				
Log SAA < 2.25	1.15 (0.66, 1.65)	< 0.001	1.14 (0.70, 1.58)	< 0.001
Log SAA ≥ 2.25	0.46 (−0.27, 1.18)	0.216	− 0.10 (−0.98, 0.78)	0.825
PWMH[a]				
Log SAA < 0.75	−0.24 (−1.53, 1.05)	0.716	−1.06 (−2.22, 0.09)	0.074
0.75 ≤ Log SAA < 1.99	1.44 (0.50, 2.38)	0.003	1.98 (1.12, 2.84)	< 0.001
Log SAA ≥ 1.99	0.09 (−0.64, 0.82)	0.800	−0.19 (−0.93, 0.55)	0.612
DWMH[a]				
Log SAA < 2	0.45 (0.17, 0.74)	0.002	0.46 (0.19, 0.74)	0.001
Log SAA ≥ 2	0.26 (−0.13, 0.65)	0.199	−0.15 (−0.59, 0.28)	0.499

Crude: no adjustment

SAA serum amyloid A protein, *PVWMH* periventricular white matter hyperintensity, *DWMH* deep white matter hyperintensity

[a]These variables were transformed to square root scale

[b]Adjusted for sex, age, hypertension, diabetes, hyperlipidemia, stroke, current smoking, antiplatelet medication, statins, total cholesterol, creatinine, homocysteine

cells, as well as stimulated endothelial cell migration and proliferation, resulting in functional suppression of the BBB [38–40]. The functional collapse of the BBB may result in the perivascular infiltration of hazardous elements (e.g., protease, immunoglobulin, and complement components) into brain tissues. These events would lead to the formation and development of PVWMH [3, 41].

In contrast, postmortem examination of the DWMH reveals more multiple tiny vacuolations, varied axonal loss, and increased tissue loss in more severe lesions, indicating potential arteriosclerosis and microcystic infarction in addition to demyelination and gliosis [4, 29, 31]. Although the particular processes are unknown, there is substantial evidence that SAA plays a causal role in atherosclerosis [13]. Initially, SAA can activate the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome in macrophages, which may explain its range of effects [42]. Furthermore, we discovered that the presence of SAA on apoB-lipoproteins increased their proteoglycan binding affinity [43], which is an important step in

the start of atherosclerosis [44]. A retrospective clinical study found that SAA levels were highly linked to blood coagulability and thrombosis [45]. In summary, subcortical areas hypoperfusion caused by atherosclerosis would eventually result in DWMH. It can be concluded that higher SAA levels have different impacts on PVWMH and DWMH via various mechanisms, which is consistent with the findings of our multivariate regression analysis.

A recent cross-sectional investigation revealed that the WMH volume, particularly PVWMH, follows a nonlinear quadratic polynomial model with a remarkable elevation between the ages of 50 and 60 years [46]. In women, the relationship between serum-free hemoglobin level and PVWMH volume was U-shaped [47]. These findings imply that there is no simple linear relationship between WMH volume and its associated risk factors. As far as we know, our study also found a consistent and independent relationship between the SAA level and PVWMH volume, which is also a nonlinear relationship. According to the two-piecewise linear regression model, SAA levels ranging

from 2.12 to 19.82 mg/L demonstrated a significant correlation with PVWMH volume. This highlights the idea that comparing SAA levels to standard WMH risk factors screening could give a more accurate means of identifying patients at risk for WMH.

Although our research incorporates several novel discoveries, we acknowledge that our study has some limitations. First, SAA, as an inflammatory marker, may drive the neuroinflammatory response after acute ischemic stroke. In a group of 1176 stroke-free subjects drawn from the same underlying population as part of a prospective cohort study among stroke patients, the mean log (SAA) was not higher among stroke patients than stroke-free participants. Moreover, there was no evidence of a time trend in levels of SAA during the 28 days of follow-up. The random effect model has demonstrated that SAA level was not associated with stroke severity, which was also consistent with the results of our sensitivity analysis [48]. In summary, first, the SAA level was hardly affected by acute ischemic stroke. In comparison, we acknowledge that the evaluation of SAA level in healthy individuals with WMH burden should have provided a better interpretation of the presumed association. Second, while we have described relationships between SAA levels and cerebral WMHs, we lack conclusive evidence that increased SAA is a risk factor for WMH burden. Also, because our study is cross-sectional and does not infer causation, future prospective studies are needed to address this issue. Third, our sample size of 179 participants was rather smaller in comparison to previous research on WMH volume and other risk variables. Therefore, after we reached our conclusion, we used the simulated statistical efficiency module of the EmpowerStats software to calculate whether the sample size was reasonable. The results of statistical analysis demonstrated that the sample size of the current study may not be optimal, but should be sufficient to draw a conclusion that may guide clinical practice (see Fig. S1 in the electronic supplementary material for details). Fourth, our study was absence of a control arm, which cannot eliminate the influence of relevant confounding factors. Next, we will conduct a 3-year prospective cohort study in a healthy population to assess whether SAA level is an independent risk factor for WMH burden. Fifth, we cannot rule out the possibility that high SAA levels are an epiphenomenon of other pathologic conditions such as possible infection or a chronic inflammatory state. Although individuals with suspicious clinical infections or inflammatory diseases were excluded, the selection bias remains. Finally, it may also have affected the concentration that the collection time point of SAA was different for different patients. To remove this effect, we attempted to minimize the difference in collection time point. However, this factor should be considered.

CONCLUSION

We demonstrated that a higher SAA level is independently associated with the development of WMH in an acute ischemic stroke group. Furthermore, these correlations were detected in a dose-dependent manner within a limited range of SAA levels. These findings, therefore, point the way forward for future research into the pathophysiology of WMH and potentially cerebrovascular diseases. Nevertheless, our conclusions must be confirmed in larger prospective studies in the future.

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Disclosures. Peng Zhang, Rongrong Han, Aimei Zhang, Xiaohong Zhang, Ziheng Zhang, HaoYu, Hongfang Li, Ziyou Qi, Peng Xu, Peng Yang, and Daojing Li declare that they have nothing to disclose regarding the content of this article.

Compliance with Ethics Guidelines. This study was performed in compliance with the Helsinki Declaration of 1964 and its later amendments. Ethics approval was obtained from the Scientific Research Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China) (approval no. 2022C075). All participants provided written informed consent regarding the use of their clinical data and biological samples for scientific research.

Data Availability. Data supporting the findings of this study are available from the corresponding author on reasonable request.

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